
THE *RONDELETIA* COMPLEX (RUBIACEAE): AN ATTEMPT TO USE ITS, *RPS16*, AND *TRNL-F* SEQUENCE DATA TO DELIMIT GUETTARDEAE, RONDELETIEAE, AND SECTIONS WITHIN *RONDELETIA*¹

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ABSTRACT

In the present study, a molecular phylogeny of the *Rondeletia* L. complex (Rubiaceae, Rondeletieae) was constructed with the following main objectives: (1) to evaluate the sections of *Rondeletia* proposed by Fernández Zequeira; (2) to test if *Stevensia* Poit. belongs to the Rondeletieae s. str.; (3) to check if ITS data from Rondeletieae support previous phylogenetic results from *trnL-F* data regarding circumscription of Rondeletieae; and (4) to verify if *Hodgkinsonia* F. Muell. belongs to Guettardeae or elsewhere. Two analyses were performed, one with ITS sequences from 46 taxa in the Rondeletieae–Guettardeae complex, and the other with combined ITS, *rps16*, and *trnL-F* sequences of 21 taxa. Representatives of nine of the 10 sections of *Rondeletia* recognized by Fernández Zequeira were included in the ITS analysis. Five of her sections could be tested for monophyly. Support was only found for *Rondeletia* sect. *Leoninae* M. Fernández Zeq., while representatives from section *Chamaebuxifoliae* M. Fernández Zeq., section *Hypoleucae* Standl., and section *Nipenses* M. Fernández Zeq. together form a well-supported clade that could be distinguished also based on morphology. The latter clade is sister to *Stevensia*, which is thus placed within *Rondeletia* s. str. In addition, ITS sequence data confirm the separation of *Rovaeanthus* Borhidi from *Rogiera* Planch. Support is low for inclusion of *Blepharidium* Standl., *Mazaea* Krug & Urb., *Phyllomelia* Griseb., *Rachicallis* DC., part of *Rogiera*, and *Suberanthus* Borhidi & M. Fernández Zeq. in Rondeletieae. *Rachicallis*, *Mazaea*, and *Phyllomelia* form a clade with strong support. The tribe Rondeletieae s. str. was found to be monophyletic in all trees, although with low support; however, a re-delimitation of the tribe is proposed here based on this study and previous phylogenetic analyses. The monophyly for the tribe Guettardeae is weakly supported, with the inclusion of *Arachnothryx* Planch. (including *Cuatrecasiodendron* Steyerem.), *Gonzalagunia* Ruiz & Pav., *Hodgkinsonia*, and *Timonius* DC. Although it was recently the subject of a molecular phylogenetic study, the tribe Guettardeae is still in need of a wide-ranging survey in order to confirm its monophyly and delimit its taxonomic boundaries. Because *Cuatrecasiodendron* was found within the *Arachnothryx* clade, the two genera are here synonymized as *Arachnothryx*, and in turn positioned within the tribe Guettardeae. In addition, based on herbarium and field studies, the two species described under *Cuatrecasiodendron* (*C. spectabile* Steyerem. and *C. colombianum* Standl. & Steyerem.) are treated as synonyms to the new combination *Arachnothryx spectabilis* (Steyerem.) Rova, Delprete & B. Bremer, which is proposed here.

Key words: *Arachnothryx*, *Cuatrecasiodendron*, Guettardeae, *Hodgkinsonia*, ITS, phylogeny, *Rogiera*, *Rondeletia*, Rondeletieae, *rps16*, Rubiaceae, *Stevensia*, *trnL-F*.

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The tribe Rondeletieae (Rubiaceae, Cinchonoideae) includes predominantly shrubs and trees and is mostly distributed in the New World tropics (Robbrecht, 1988; Delprete, 1999a), with the main center of diversity in the Greater Antilles. A thorough description of the taxonomic and systematic history of the tribe is found in Delprete (1999a).

The largest genus of the tribe, *Rondeletia* L., is mainly Antillean and comprises approximately 120 species. Standley (1918) divided *Rondeletia* into 15 sections based on morphological and distributional data. Since then, several morphological and molecular studies in the Rondeletieae have argued about the status of Standley's sections and the circumscription of the genus *Rondeletia*. One opinion is that *Rondeletia* should be regarded as a narrowly circumscribed genus, separated from morphologically similar genera such as *Arachnothryx* Planch., *Javorkaea* Borhidi & Jarai-Koml., *Rogiera* Planch., *Roigella* Borhidi & M. Fernández Zeq., *Rovaeanthus* Borhidi, and *Suberanthus* Borhidi & M. Fernández Zeq. (Steyermark, 1967; Borhidi & Fernández Zequeira, 1981a, b; Borhidi, 1982, 1989, 1994; Borhidi & Járαι-Komlódi, 1983; Fernández Zequeira, 1994; Delprete, 1999a, as *Rondeletia* complex sensu Delprete; Rova, 1999; Rova et al., 2002; Borhidi et al., 2004; Rova, unpublished). On the other hand, Lorence (1991) recognized *Rondeletia* as a widely circumscribed genus, treating the names applied to Mexican and Central American taxa of the complex as synonyms. Based on morphological data, Fernández Zequeira (1994) made an attempt to classify the Greater Antillean (especially the Cuban) *Rondeletia* species into 10 sections. Her classification comprised 104 species, most of them endemic to Cuba. This means that a majority of the species of *Rondeletia* s. str. were included in her study. According to Fernández Zequeira (1994), the sections are distinguished by various combinations of (often multistate) morphological characters such as position and shape of inflorescence, flower merosity, calyx lobe shape, leaf indumentum, and phyllotaxy (leaves opposite vs. verticillate). However, her focus on Cuban species did little to resolve the problem in the larger *Rondeletia* complex. The first aim of the present study was to test if Fernández Zequeira's sections of *Rondeletia* are supported by phylogenies obtained from molecular sequences. The second aim was to test if *Stevensia* Poit. was closely related to *Rondeletia* or not. *Stevensia* has not been included previously in molecular phylogenies, but morphology suggests a close affinity between the genera. Earlier studies (Bremer et al., 1995; Bremer & Thulin, 1998; Andersson & Rova, 1999; Rova et al., 2002) have shown that the tribes Guettardeae and Rondeletieae

are closely related, and this study also aimed to investigate if ITS data would suggest a similar circumscription of Rondeletieae as previous studies had. Finally, the study was aimed to investigate if ITS sequence data would support Bremer's (1992) inclusion of the Australian genus *Hodgkinsonia* F. Muell. in the Guettardeae or Delprete's (1996) transfer of the genus from the Guettardeae to the Chiococceae.

MATERIALS AND METHODS

For the ITS analyses, material was sampled from as many *Rondeletia* species and subspecies as possible. An effort was made to include representatives from all genera in Rondeletieae sensu Rova et al. (2002). The outgroup consisted of *Luculia* Sweet (basal in Rubiaceae, e.g., Bremer et al., 1995), *Catesbaea fuertesii* Urb., *Chiococca alba* (L.) Hitchc. (Chiococceae s.l.), and 12 accessions representing 11 species in the following six genera of the tribe Guettardeae (based on available material and the results from Rova et al., 2002): *Arachnothryx*, *Cuatrecasiodendron* Steyermark., *Gonzalagunia* Ruiz & Pav., *Guettarda* L., *Rogiera*, and *Timonius* DC. Authors of species names are given in Table 1, or otherwise when first mentioned in the text.

Fresh or silica gel-dried leaves were used for DNA extraction when available, but often herbarium material had to be used. DNA was extracted using the CTAB method (Doyle & Doyle, 1987) and cleaned with the QIAquick PCR Purification Kit (QIAGEN GmbH, Hilden, Germany). The cocktail for polymerase chain reaction (PCR) amplification was mixed as follows (to ca. 25 µl): 2.5 µl 10× buffer, 2.5 µl 25 mM MgCl₂, 2 µl dNTP, 0.125 µl Taq DNA polymerase, 0.625 µl 10 µM forward primer, 0.625 µl 10 µM reverse primer, 2.5 µl 0.1 M TMACl, 2 µl dimethyl sulfoxide (DMSO), 2 µl template, and 10 µl water. In some cases, the amount of primer or template was doubled (replacing some of the water). Primers P17 and 26S-82R (Popp & Oxelman, 2001) were used for amplification. Sequencing reactions were realized using the DYEnamic ET terminator Cycle Sequencing kit (Amersham Biosciences, Buckinghamshire, England) following the protocol of the manufacturer (DMSO was added in the same concentration as in the PCR mix) and run on a MegaBACE 1000 DNA Analysis System (Amersham Biosciences). For sequencing, the same primers were used as in the PCR amplification.

For the ITS study, 50 new ITS sequences were produced, and five additional sequences were downloaded from GenBank and included in the data matrix. Taxon names, authors, vouchers, and GenBank accession numbers are presented in Table 1.

Manual alignment and gapcoding of the ITS sequences were performed with the following criteria: (1) an effort was made to see if gaps/insertions could be interpreted as caused by repeats or inversions, and if so, sequences were aligned to fit these possible events; (2) gaps (i.e., inferred insertion/deletion events) were introduced into the sequences to keep the number of substitutions in an aligned region to a minimum; (3) insertions/deletions and substitutions were considered equally probable events; and (4) gaps/insertions of equal length shared by two or more taxa were inferred to be homologous and binary coded. Gaps of more than one position in length introduced due to multiplication of single nucleotides, e.g., poly-A, were not coded. Regions where alignment could not be unambiguously interpreted were removed from the analysis. After alignment, two ITS matrices were produced, one including gap codings and the other without them. Two parsimony analyses, conducted with PAUP* version 4.0b10 (Swofford, 2000), were performed for each matrix. The first ITS analysis was a heuristic search (random addition sequence with 1000 replicates, tree bisection-reconnection [TBR] branch swapping, and MULTREES option in effect), and the second analysis was a jackknife search (faststep search option, 10,000 replicates, and Jac resample emulation).

For the combined analyses, the data matrix from the ITS study was combined with the entire matrices from the *trnL-F* study of Rova et al. (2002) and from an *rps16* analysis (Rova, unpublished), keeping the indel codings from each matrix. Previous analyses of each separate data set resulted in similar trees, which implied that the data sets were congruent. Taxa not included in the combined analyses were then deleted using the command DELETE in the PAUP block. The resulting set of sequences comprised 20 ingroup taxa. This set included all taxa where all three sequences were available and all *Rondeletia* species where at least ITS and *rps16* sequences were available. *Chiococca alba* was used as outgroup, because, previously, it had been clearly shown not to be part of the ingroup (Rova et al., 2002). The data were analyzed by a heuristic search (random addition sequence with 1000 replicates, TBR branch swapping, and MULTREES option in effect).

RESULTS

More than 50 DNA extractions were obtained from *Rondeletia* representatives, but only 27 of these (representing 23 species) were amplified by PCR and yielded sequences. Extractions that did not produce usable sequences were mostly made from herbarium material more than 50 years old. Material collected in silica gel almost always worked for PCR

and resulted in high-quality sequences. It was not possible to obtain sequences from section *Lindenianae* M. Fernández Zeq., although extractions were attempted from two different specimens. It was also not possible to obtain PCR products from *Roigella correifolia* (Griseb.) Borhidi & M. Fernández Zeq., which *trnL-F* data showed to be closely related to *Rondeletia* s. str. (Rova et al., 2002). For four sections (*Rondeletia* sect. *Odoratae* Standl., section *Pedicellares* Standl., section *Rigidae* M. Fernández Zeq., and section *Chamaebuxifoliae*), it was only possible to sequence one species from each section. We were unfortunately not able to sequence the type species of *Rondeletia*, *R. americana* L. This species seems to be very rarely collected, and extractions made from the herbarium material that we found in the Swedish Museum of Natural History Herbarium did not amplify despite several attempts. We were not able to establish contact with anyone who could assist us with recently collected material from St. Vincent, where the species is endemic, and it was not possible to do such fieldwork ourselves. Ten species that yielded sequences were not listed under any section in the work of Fernández Zequeira, but four of them could be assigned to sections based on the key provided in her paper (Fernández Zequeira, 1994): *R. inermis* (Spreng.) Krug & Urb. and *R. pilosa* Sw. belonging to section *Leoninae*, and *R. hameliifolia* Dwyer & M. V. Hayden and *R. purdiei* Hook. f. belonging to section *Calophyllae* M. Fernández Zeq. Sectional affinities are indicated in Figure 1.

The first ITS matrix, without indel coding, included 699 characters, of which 174 were parsimony informative. The second ITS matrix, where indels were coded, included 723 characters, of which 198 were parsimony informative. The combined ITS, *rps16*, and *trnL-F* matrix included 2751 characters, 1451 of which were parsimony informative.

The strict consensus tree obtained from the ITS analyses is presented in Figure 1. Heuristic searches of both data sets each resulted in 48 most parsimonious trees. Tree lengths were 768 (consistency index [CI] = 0.56, retention index [RI] = 0.76) in the heuristic search of the data set without indel coding and 805 (CI = 0.65, RI = 0.77) in the heuristic search where indels were coded. Strict consensus trees were identical for both data sets. Jackknife support was not found for all clades in the strict consensus trees from the heuristic searches, and jackknife support values for a clade could vary up to more than 10 units between the two data sets. Tree topologies differed only marginally between the two jackknife searches. The jackknife search without indel codings found one clade that was not found in the other jackknife search (or in the heuristic

searches), and the jackknife search with indel codings resulted in two clades not found in the jackknife search without indels coded (Fig. 1). Support for these clades was low in all cases.

The analysis of the combined matrix resulted in 12 equally parsimonious trees (length 2046, CI = 0.91, RI = 0.97). A strict consensus of these trees is shown in Figure 2, and branches from the consensus tree that also occur in the ITS analysis are marked in bold in Figure 1.

DISCUSSION

SECTIONAL CLASSIFICATION OF *RONDELETIA*

The main aim of our study was to test Fernández Zequeira's (1994) classification with 10 sections of *Rondeletia* using molecular phylogenetic analyses. This goal was hard to reach satisfactorily; despite an extensive search, it was difficult to find herbarium or silica gel-dried material that would work for PCR and sequencing. For five of the sections, only one representative of each could be sequenced. Furthermore, ITS data are obviously not variable enough to provide resolution among sections *Hypoleucae* and *Nipenses*. Nevertheless, we obtained several interesting results with regard to the circumscription of *Rondeletia* and some of Fernández Zequeira's sections.

There is strong support for the *Rondeletia* s. str. clade (Fig. 1, clade E). This clade consists of predominantly Antillean species. The only exceptions to this distribution are *R. hameliifolia* from Central America (Panama) and *R. purdiei* from South America (Ecuador). Neither *R. hameliifolia* nor *R. purdiei* were included in Fernández Zequeira's (1994) treatment, but according to her identification key, both species would belong to section *Calophyllae*. In our study, the two species form a clade with strong support. A third representative of this section is *R. alaternoides* A. Rich. from Cuba, which is found in clade F (Fig. 1). Thus, ITS sequence data do not support a monophyletic section *Calophyllae*.

Rondeletia deamii (Donn. Sm.) Standl. is found just outside the *Rondeletia* s. str. clade. The generic position of this Central American species has recently been under debate. Lorence (1999) supported its position in *Rondeletia*, but Borhidi (2001a) positioned it in *Arachnothryx*. Our ITS sequence data suggest that this species should be treated as a *Rondeletia*, although support for this hypothesis is less than 50.

Rondeletia inermis and *R. pilosa* Sw. were not included in Fernández Zequeira's (1994) treatment of Cuban *Rondeletia*, as these species occur in Puerto Rico and the U.S. Virgin Islands, respectively. However, according to her key to sections, they would

both belong to *Rondeletia* sect. *Leoninae*. In our analysis, they form a clade with strong support. *Rondeletia* sect. *Leoninae* would thus be the only one of Fernández Zequeira's sections that is supported by our ITS sequence data.

In all analyses, there is moderate support for a clade with *Rondeletia alaternoides*, *R. odorata* Jacq., and *R. pachyphylla* Krug & Urb. (Fig. 1, clade F), which represent sections *Calophyllae*, *Odoratae*, and *Pedicellares*, respectively. Following the diagnostic table of sections in Fernández Zequeira (1994), we were unable to find any morphological characters that support this group.

Rondeletia intermixta Britton and *R. ochracea* Urb. form a clade with strong support. While *R. intermixta* belongs to section *Rondeletia* M. Fernández Zeq., *R. ochracea* has not been previously classified to any section. It is thus possible to argue that *R. ochracea* should also belong to section *Rondeletia*. The only other known representative of section *Rondeletia* included in our analysis, *R. portoricensis* Krug & Urb., is placed in an unresolved relationship to the *R. intermixta*–*R. ochracea* clade, although jackknife support for this is below 50.

Our study does not show any support for a separation of sections *Hypoleucae* and *Nipenses* in *Rondeletia*. All sequenced representatives of these sections, except *R. berteriana* DC., are found in a strongly supported but unresolved clade (Fig. 1, clade H). No morphological character combination seems to be unique for these two sections as one group, according to the character list in Fernández Zequeira (1994). *Rondeletia berteriana* differs from the other sequenced species of section *Hypoleucae* (and section *Nipenses*) in being from Hispaniola. This species is found as sister to clade H but with very low support (Fig. 1).

Rondeletia chamaebuxifolia Griseb., the only sequenced representative of section *Chamaebuxifoliae*, is found closely related to the species from sections *Hypoleucae* and *Nipenses*. Following the diagnostic characters provided in Fernández Zequeira (1994) for sections *Chamaebuxifoliae*, *Hypoleucae*, and *Nipenses*, this clade (Fig. 1, clade G) could be distinguished from other sections by having 1- to 3-flowered inflorescences and retrorse-pilose flowers.

STEVENSIA

The second aim of our study was to investigate the relationships between *Stevensia* and *Rondeletia*. *Stevensia* is here for the first time included in a molecular phylogenetic study. According to ITS data, there is strong support for an inclusion of at least *S. minutifolia* Alain in *Rondeletia* s. str. The genus

Table 1. Table of taxa included in the study, including ITS and *rps16* voucher information for sequences originally presented in this paper, as well as GenBank accession numbers for all sequences used. If only one voucher is mentioned, the same voucher is used for both ITS and *rps16* sequencing unless the other sequence is cited from a previous publication.

Taxon	Voucher data	GenBank accession number		
		ITS	<i>rps16</i>	<i>trnL-F</i>
<i>Acrosynanthus revolutus</i> Urb.	Cuba, P. G. Delprete et al. 8818 (UPS)	AY730288		
<i>Arachnohryx buddleioides</i> (Benth.) Planch.	Panama, J. Rova & Sundbaum 2411 (S)	AY730299		
<i>Arachnohryx chimboracensis</i> (Standl.) Steyerem.	Ecuador, P. G. Delprete & A. Verduga 6398 (NY)	AY730292		
<i>Arachnohryx leucophylla</i> (Kunth) Planch.	Cuba, J. Rova et al. 2287 (GB)	AY730296	AF242910	AF152718 ⁶
<i>Arachnohryx</i> sp. indet.	Ecuador, G. Harling 27108 (NY)	AY730298		
<i>Blepharidium guatemalense</i> Standl.	Guatemala, Gustafsson et al. 212 (GB)	AY730287	AF242916	AF152735 ⁶
<i>Catesbaea fuertesii</i> Urb.		AY205364 ¹		
<i>Chiococca alba</i> (L.) Hitchc.		AY205367 ¹	AF004034 ⁴	AF102400 ⁷
<i>Cuatrecasasiodendron spectabile</i> Steyerem.	Colombia, J. Rova et al. 2093 (S)	AY730297	AF242934	
<i>Gonzalagunia affinis</i> Standl. ex Steyerem.	Ecuador, Bremer et al. 3350 (UPS)	AY730295		
<i>Guettarda scabra</i> (L.) Lam.	Cuba, Rova et al. 2260 (GB)	AF323061 ²	AF242963	
<i>Guettarda uruguensis</i> Cham. & Schltdl.	cultivated at Fairchild Tropical Garden, FTC x 5-127, Gillis 9575 (FTG)	AY730294		
<i>Hodgkinsonia ovatiflora</i> F. Muell.	Australia, Puttock 8602667 (UNSW)	AY730293		
<i>Luculia grandifolia</i> Ghose		AJ346896 ³		
<i>Mazaea phialanthoides</i> (Griseb.) Krug & Urb.	Cuba, J. Rova et al. 2264 (GB)	AY730302	AF242980	AF152749 ⁶
<i>Mazaea shaferi</i> (Standl.) Delprete	ITS: Cuba, T. McDowell 4826-1993 (DUKE); <i>rps16</i> : Cuba, Rova et al. 2224 (GB)	AY730304	AF242911	AF152750 ⁶
<i>Phyllomelia coronata</i> Griseb.	Cuba, P. G. Delprete et al. 8913 (UPS)	AY730303		
<i>Rachicallis americana</i> (Jacq.) Hitchc.	cultivated at Fairchild Tropical Garden, FTC 64-266, Fanning KF81 (FTG)	AY730301	AF004073 ⁴	AF152747 ⁶
<i>Rogiera amoena</i> Planch.	ITS: Mexico, Mexia 8982 (GB); <i>rps16</i> : Guatemala, Hawkes et al. 1962 (S)	AY730286	AF243000	AF102474 ⁷
<i>Rogiera cordata</i> (Benth.) Planch.	ITS: Guatemala, Gustafsson & Fredriksson 126 (GB); <i>rps16</i> : cultivated at Royal Botanical Garden, Kew, Chase 2267 (K)	AY730285	AF242999	AF152715 ⁶
<i>Rogiera cordata</i> (Benth.) Planch.	Guatemala, J. J. Castillo & R. Luarca 2005 (NY)	AY730284		
<i>Rovaeanthus strigosus</i> (Benth.) Borhidi	cultivated at PTBG, D. Lorence 8920 (PTBG)	AY730291		
<i>Rovaeanthus suffrutescens</i> (Brandegee) Borhidi	cultivated at the Bergius Botanical Garden, Stockholm, Bremer 2712 (S)	AY730290	AF243003	AF152738 ⁶
<i>Rondeletia alaternoides</i> A. Rich. subsp. <i>alaternoides</i>	Cuba, P. G. Delprete et al. 8725 (UPS)	AY730306		
<i>Rondeletia alaternoides</i> subsp. <i>brachyloba</i> M. Fernández Zeq. & Borhidi	Cuba, J. Rova et al. 2228 (GB)	AY730310	AF243006	AF152740 ⁶

Table 1. Continued.

Taxon	Voucher data	GenBank accession number		
		ITS	<i>rps16</i>	<i>trnL-F</i>
<i>Rondeletia apiculata</i> Urb.	Cuba, J. Rova et al. 2243 (GB)	AY730312	AF243007	
<i>Rondeletia barahonensis</i> Urb.	Dominican Republic, P. G. Delprete et al. 7534 (UPS)	AY730321		
<i>Rondeletia berteriana</i> DC.	Dominican Republic, P. G. Delprete et al. 7567 (UPS)	AY730322		
<i>Rondeletia chamaebuxifolia</i> Griseb.	Cuba, Machado et al. s.n. 1995-03-16 (S)	AY730327		
<i>Rondeletia cincta</i> Griseb.	Jamaica, P. G. Delprete et al. 7503 (UPS)	AY730323		
<i>Rondeletia</i> sp.	Jamaica, P. G. Delprete et al. 7479 (UPS)	AY730324		
<i>Rondeletia deamii</i> (Donn. Sm.) Standl.	Guatemala, J. J. Castillo 1560 (NY)	AY730305	AJ786765 ⁵	
<i>Rondeletia hameliifolia</i> Dwyer & M. V. Hayden	Panama, J. H. Kirkbride & S. M. V. Hayden 164 (NY)	AY730326		
<i>Rondeletia inermis</i> (Spreng.) Krug & Urb.	ITS: Cultivated at PTBG, D. Lorence 8796 (PTBG); <i>rps16</i> : Puerto Rico, Acevedo-Rodriguez et al. 7691 (NY)	AY730315	AF243012	AF152745 ⁶
<i>Rondeletia intermixta</i> Britton subsp. <i>intermixta</i>	Cuba, J. Rova et al. 2245 (GB)	AY730311	AF004077 ⁴	AF152742 ⁶
<i>Rondeletia lomensis</i> Urb.	Cuba, J. Rova et al. 2216 (GB)	AY730313		
<i>Rondeletia miraflorensis</i> M. Fernández Zeq. & Borhidi	Cuba, J. Rova et al. 2217 (GB)	AY730314	AF243009	
<i>Rondeletia nipensis</i> Urb.	Cuba, P. G. Delprete et al. 8651 (UPS)	AY730330		
<i>Rondeletia nipensis</i> subsp. <i>moaensis</i>	Cuba, P. G. Delprete et al. 8770 (UPS)	AY730325		
<i>Rondeletia ochracea</i> Urb.	Dominican Republic, W. C. Holmes et al. 6618 (NY)	AY730316		
<i>Rondeletia odorata</i> Jacq.	cultivated at the Bergius Botanical Garden, Stockholm, Bremer & Andreasen 3504 (UPS)	AY730307	AF243010	AF152741 ⁶
<i>Rondeletia pachyphylla</i> Krug & Urb. subsp. <i>myrtilloides</i> M. Fernández Zeq. & Borhidi	Cuba, J. Rova et al. 2232 (GB)	AY730331	AF243011	
<i>Rondeletia pachyphylla</i> Krug & Urb. subsp. <i>pachyphylla</i>	Cuba, P. G. Delprete et al. 8674 (UPS)	AY730317		
<i>Rondeletia pilosa</i> Sw.	U.S. Virgin Islands, P. Acevedo-Rodriguez 2836 (NY)	AY730332	AF243014	AF152744 ⁶
<i>Rondeletia pitreana</i> Urb. & Ekman	Hispaniola, Liogier 13966 (NY)	AY730289		
<i>Rondeletia plicatula</i> Urb.	Cuba, P. G. Delprete et al. 8716 (UPS)	AY730318		
<i>Rondeletia portoricensis</i> Krug & Urb.	Puerto Rico, C. M. Taylor 11687 (MO)	AY730333	AF243015	AF152743
<i>Rondeletia purdiei</i> Hook. f.	Ecuador, Corbisier-Baland 1905 (UPS)	AY730328		
<i>Rondeletia stipularis</i> (L.) Druce	Jamaica, P. G. Delprete et al. 7472 (UPS)	AY730319		
<i>Rondeletia subcanescens</i> M. Fernández Zeq. & Borhidi	Cuba, P. G. Delprete et al. 8761 (UPS)	AY730320		
<i>Rondeletia subcanescens</i> M. Fernández Zeq. & Borhidi	Cuba, P. G. Delprete et al. 8833 (UPS)	AY730329		
<i>Stevensia minutifolia</i> Alain	Dominican Republic, P. G. Delprete et al. 7540 (UPS)	AY730308		
<i>Stevensia minutifolia</i> Alain	Dominican Republic, A. Liogier 13663 (NY)	AY730309		
<i>Suberanthus brachycarpus</i> (Griseb.) Borhidi & M. Fernández Zeq.	Cuba, T. McDowell 4824-1993 (DUKE)	AY730300		
<i>Timonius nitidus</i> Fern.-Vill.		AF323063 ²		

The cited literature for published sequences includes: ¹McDowell et al., 2003; ²Moynihan & Watson, 2001; ³Razafimandimbison & Bremer, 2002; ⁴Andersson & Rova, 1999; ⁶Rova et al., 2002; ⁷Struwe et al., 1998. Sequences were also taken from ⁵S. Stranczinger, F. Jakab, J. L. Szentpeteri & A. Borhidi, unpublished.

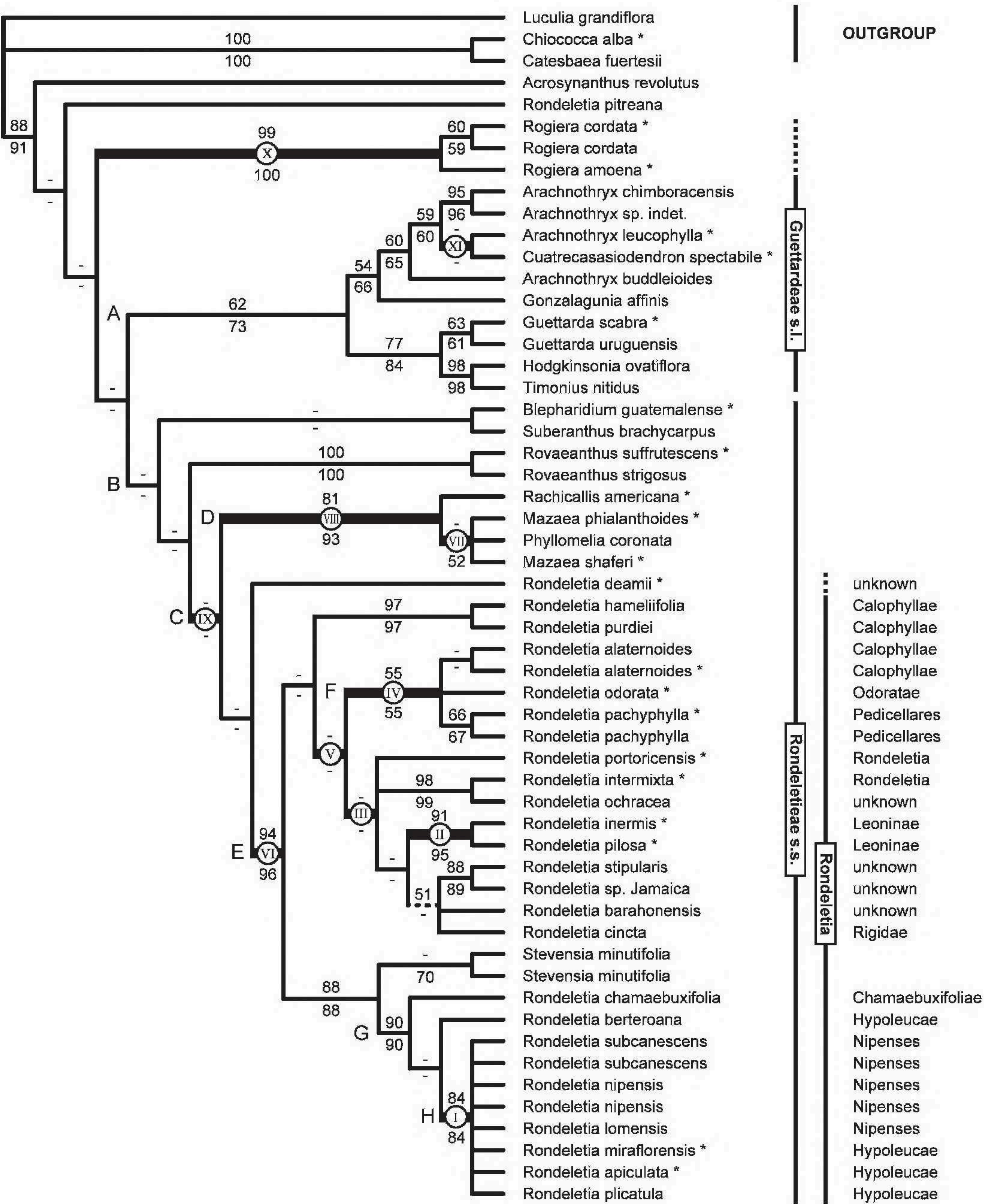


Figure 1. Tree compiled from the strict consensus trees from the two heuristic searches, without and with indels coded, respectively. The one dotted branch was not found in the heuristic searches, only in the jackknife search without indels coded. Numbers indicate jackknife support: numbers above branches are support values without indels coded, and numbers below branches are support values with indels coded. Jackknife support of 50 and lower is indicated by dashes (–). Letters A–H indicate the clades discussed in the text: —A. Clade comprising representatives of the tribe Guettardeae. —B. Clade corresponding to Rondeletieae sensu Rova et al. (2002). —C. Clade comparable to the one in which *Acrosynanthus* was found in Rova et al. (2002). —D. Clade comprising *Mazaea*, *Phyllomelia*, and *Rachicallis*. —E. The *Rondeletia* s. str. clade. —F. Clade including representatives of sections *Odoratae*, *Pedicellares*, and the paraphyletic section *Calophyllae*. —G. Clade distinguished by having 1- to 3-flowered inflorescences and retrorse-pilose flowers (corresponding to sections *Chamaebuxifoliae*, *Hypoleucae*, and *Nipenses*). —H. Clade comprising Cuban representatives of sections *Hypoleucae* and *Nipenses*. Taxa marked by an asterisk (*) are included in the combined ITS, *rps16*, and *trnL-F* analysis presented in Figure 2. The circled Roman numerals I–XI on bold branches refer to clades in the combined ITS, *rps16*, and *trnL-F* analysis (Fig. 2).

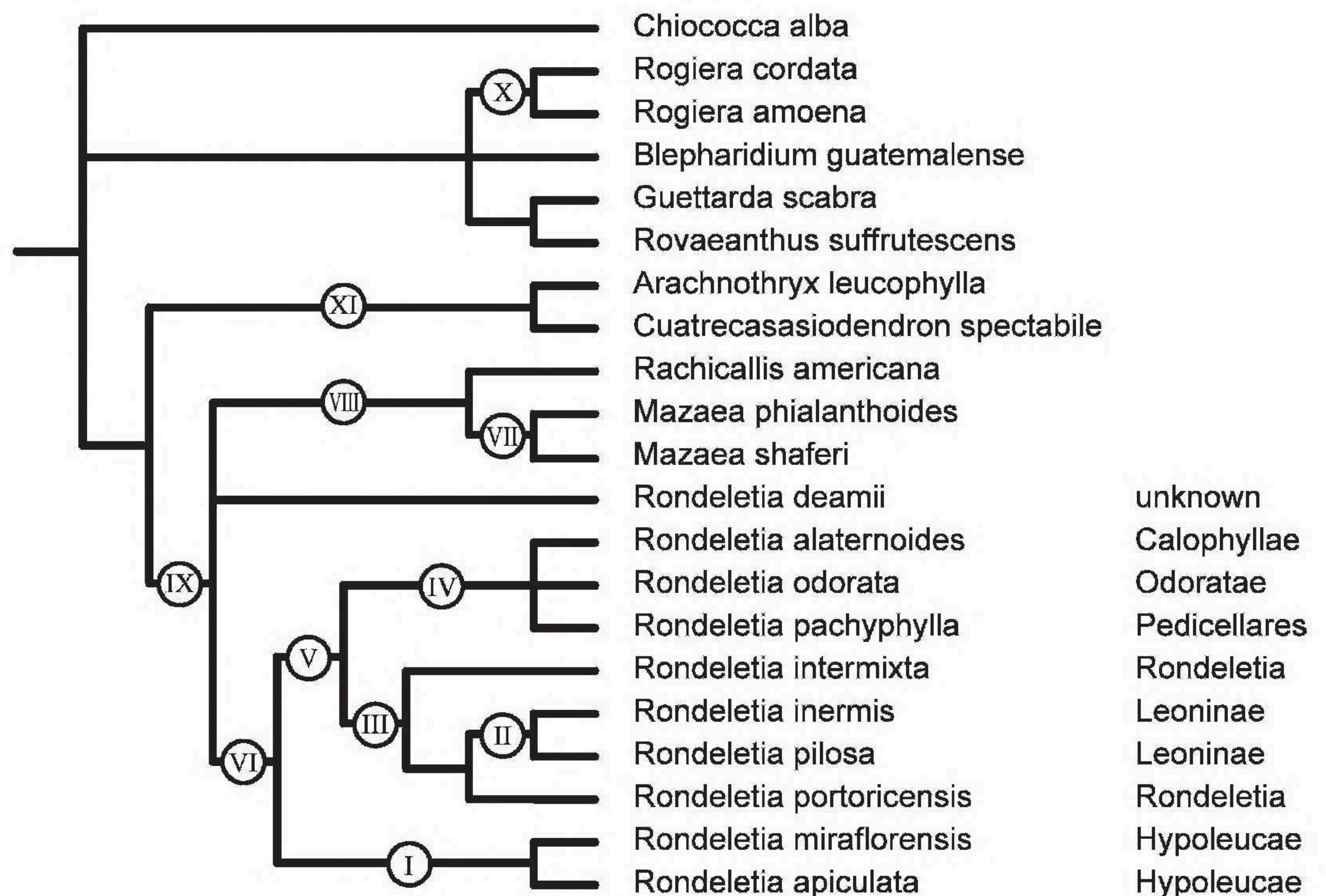


Figure 2. Strict consensus tree of the 12 equally parsimonious trees from the analysis of the combined ITS, *rps16*, and *trnL-F* data matrix. *Guettarda scabra*, *Cuatrecasasiodendron spectabile*, *Rondeletia deamii*, *R. pachyphylla*, *R. miraflorensis*, and *R. apiculata* were only represented by ITS and *rps16* data in the data set. The circled Roman numerals I–XI on branches refer to clades in the tree in Figure 1. Sectional assignment of *Rondeletia* species, according to Fernández Zequeira (1994), is listed in the rightmost column. *Rondeletia inermis* and *R. pilosa* were not listed in Fernández Zequeira (1994), but have been assigned to section based on her key. *Rondeletia deamii* is not assigned to a section (cf. unknown among the sectional assignments).

Stevensia comprises 11 species endemic to Hispaniola. It is recognized by triangular stipules connected to a sheath, solitary and axillary flowers, two to three calyx lobes, five to seven stamens attached in the corolla throat, glabrous style, and ovoid to oblong seeds (Borhidi, 2001b). However, several of these character states are also found within *Rondeletia* s. str., according to Fernández Zequeira (1994). We therefore suggest that *Stevensia* should be included within *Rondeletia* s. str., pending future studies with an extended sampling.

GUETTARDEAE AND RONDELETIEAE

Our third aim was to compare a nuclear ITS phylogeny of the Rondeletieae with the results from a previous *trnL-F* chloroplast DNA (cpDNA) study

(Rova et al., 2002). The fourth aim was to see if ITS sequence data would place *Hodgkinsonia* in Guettardeae or elsewhere.

Acrosynanthus revolutus Urb. and *Rondeletia pitreana* Urb. & Ekman (not classified to section) appear as early diversified lineages in the cladogram. One possible explanation for the position of *R. pitreana* could be that we were not able to read the sequence in its entirety. Because of this, it is about 40 bases shorter than the other *Rondeletia* sequences. Another possibility is that *R. pitreana* does not belong to *Rondeletia*. In any case, further studies are needed to solve the position of *R. pitreana*. The position of *Acrosynanthus* Urb. in the present analysis differs markedly from the results of the *trnL-F* study from Rova et al. (2002), where *Acrosynanthus* was found in a position equivalent to basal in clade C (Fig. 1). A

←

Sectional assignment of *Rondeletia* species, according to Fernández Zequeira (1994), is listed in the rightmost column. *Rondeletia hameliifolia*, *R. purdiei*, *R. inermis*, and *R. pilosa* were not listed by Fernández Zequeira, but have been assigned to section based on her key (1994: 106).

possible explanation would be that *Acrosynanthus* is not monophyletic: *A. latifolius* Standl. was included in the *trnL-F* study, but *A. revolutus* was sequenced in the ITS analysis. However, the possible paraphyly of *Acrosynanthus* must be left to another study when more material of this genus is available.

We found that the well-supported Guettardeae s.l. and Rondeletiae s. str. clades in the *trnL-F* study from Rova et al. (2002) have only weak support from ITS sequence data.

With regard to the tribe Guettardeae, there is moderate support for a clade including *Arachnothryx*, *Gonzalagunia*, *Cuatrecasasiendron*, *Guettarda*, *Hodgkinsonia*, and *Timonius* (Fig. 1, clade A). According to our results, *Cuatrecasasiendron* should be synonymized with *Arachnothryx*, and this is also morphologically supported (see taxonomic treatment below). While *trnL-F* data (Rova et al., 2002) showed *Rogiera amoena* Planch. and *R. cordata* (Benth.) Planch. as members of the Guettardeae, the inclusion of *Rogiera* s. str. in the Guettardeae clade is not supported by ITS data alone. In the combined analysis (Fig. 2), *Rogiera* is found within Guettardeae, while *Arachnothryx* is found to be more closely related to Rondeletiae.

In a recent molecular phylogenetic study, Achille et al. (2006) supported the monophyly of the Guettardeae as recognized here, although they showed that *Guettarda*, *Antirhea* Comm. ex Juss., and *Stenostomum* C. F. Gaertn. are polyphyletic. However, more genera and more species need to be included in the study in order to test the monophyly and delimitation of this tribe.

Although Rondeletiae sensu Rova et al. (2002) is recognized by ITS data in the consensus trees (Fig. 1, clade B), there is no jackknife support for this clade.

In both heuristic ITS searches, the genera *Blepharidium* Standl. and *Suberanthus* were found basal in the Rondeletiae s. str. clade, but again, there is no jackknife support for this. However, this position corresponds to the results from the *trnL-F* study of Rova et al. (2002).

The ITS data place *Rovaeanthus strigosus* (Benth.) Borhidi in the Rondeletiae, as sister taxon to *R. suffrutescens* (Brandegge) Borhidi. Just as in the *trnL-F* study (Rova et al., 2002), the ITS data indicate that *R. suffrutescens* belong to the Rondeletiae s. str. (although this is contradicted in the combined analysis where *R. suffrutescens* is found as sister to *Guettarda*). In any case, *R. suffrutescens* always appears in a separate position from *Rogiera*, and our study thus supports the transfer of these species from *Rogiera* into a new genus as proposed by Borhidi et al. (2004).

There is strong support for a close relationship between *Rachicallis* DC., *Mazaea* Krug & Urb., and

Phyllomelia Griseb. (D in Fig. 1). *Rachicallis* and *Phyllomelia* are monotypic genera, and *Mazaea* only comprises two species. Based on ITS data, one could argue that all three genera should be merged together. However, both *Mazaea* and *Phyllomelia* are easily distinguished by the peculiar fruit (pseudosamara, sensu Delprete, 1999b) and calyx morphology (Delprete, 1999b), and for this reason we prefer to regard them as separate genera.

HODGKINSONIA

The fourth aim was to see if ITS sequence data would place *Hodgkinsonia* in Guettardeae or elsewhere. Our study undoubtedly places *Hodgkinsonia* close to *Timonius*, which means within Guettardeae. This position is in accordance with the view of Mueller (1861) in the original description and Bremer (1992), but contradicts the supposition of Delprete (1996), who tentatively included the genus in tribe Chiococceae in agreement with Robbrecht (1988).

CONCLUSION

The ITS sequence data support only one of Fernández Zequeira's (1994) *Rondeletia* sections as monophyletic: section *Leoninae*. *Rondeletia* sections *Calophyllae* and *Rondeletia* are paraphyletic according to our analysis. However, one should bear in mind that we were not able to sequence more than one species from several sections. When we compare our ITS phylogeny with the character lists in Fernández Zequeira's treatment of *Rondeletia*, we were unable to find morphological characters that correspond with our phylogenies. The sections described by Fernández Zequeira are often defined by various combinations of overlapping character states, which makes comparisons difficult. The only exception is a clade including representatives from *Rondeletia* sections *Chamaebuxifoliae*, *Hypoleucae*, and *Nipenses*, which could be distinguished by having 1- to 3-flowered inflorescences and retrorse-pilose flowers. This clade could potentially be recognized as one section.

Rondeletia s. str. (i.e., excluding *Arachnothryx*, *Javorkaea*, *Rogiera*, *Roigella*, *Rovaeanthus*, and *Suberanthus*) has strong support, although some species need to be further investigated for their generic affinity (e.g., *R. pitreana* and *R. deamii*).

An important result from our study is that *Stevensia minutifolia* is included within *Rondeletia* s. str. A reevaluation of the morphological characters in *Rondeletia* (including *Stevensia*) based on the results from ITS and other sequence data is certainly needed. The present analysis clearly suggests that *Stevensia* should be recognized at most as a section of *Rondeletia*.

There is strong support for a division of the Rondeletieae–Guettardeae complex into the tribes Rondeletieae s. str. and Guettardeae s.l. from *trnL-F* data (Rova et al., 2002), but only weak support from ITS data. While part of the Guettardeae has moderate support, support for Rondeletieae in the sense of Rova et al. (2002) is weak in the ITS study, although the Rondeletieae s. str. clade is found in the strict consensus trees of all of our analyses, both including and excluding indel codings. It was not possible to compile a sufficiently large data set in order to test the delimitations of Guettardeae and Rondeletieae using a combined ITS, *rps16*, and *trnL-F* sequence data matrix; however, we consider that the phylogenies available up to this point (Rova, 1999; Rova et al., 2002; Delprete & Cortés-B., 2004; the present study) provide sufficient support for a re-delimitation of the tribe Rondeletieae.

Based on the results from ITS sequence data, we also reconsider Delprete's (1996) tentative inclusion (based on morphology) of *Hodgkinsonia* in the Chio-cocceae, since the present ITS sequence data support Bremer's (1992) conclusion (also based on morphological data) that *Hodgkinsonia* is part of the tribe Guettardeae.

TAXONOMIC TREATMENT

Based on the results from the present and other recent studies (Delprete, 1999b; Rova, 1999; Rova et al., 2002; Delprete & Cortés-B., 2004; Borhidi et al., 2004), we propose the following taxonomic descriptions and rearrangements.

Tribe **Rondeletieae** (DC.) Miq., Flora Nederl. Indië 2: 130, 156. 1856. Rondeletiinae DC., Prodr. 4: 342, 401. 1830, as subtribe "Rondeletieae," tribe Hedyotideae. Rondeletieae DC. ex Rchb., Der Deutsche Botaniker 1: 77. 1841, stat. non indic. TYPE: *Rondeletia* L.

Shrubs or trees; wood whitish or yellowish; raphides absent; axillary thorns absent. Stipules free or connate at base, mostly entire, rarely bifid, mostly interpetiolar, frequently with colleters on the adaxial side secreting resinous compounds, persistent to readily caducous; leaves opposite or verticillate, decussate, petiolate to sessile, blades chartaceous to thick-coriaceous; domatia variably present or absent. Inflorescences terminal or axillary, cymose, paniculate or thyrsoid, multiflorous or pauciflorous, or uniflorous. Flowers hermaphroditic, mostly actinomorphic, (3- to)4- to 6-merous; calyx persistent or caducous; lobes often minute, sometimes foliose; calycophylls commonly absent or pterophyllous (green to greenish white), with all calyx lobes expanding into

a rotate pterophyll after anthesis and present in all flowers in *Phyllomelia*; corolla hypocrateriform or narrowly infundibuliform, orifice with annular thickening, white, cream-white, red, green, or yellow, membranous to fleshy; aestivation valvate, contorted, or imbricate; stamens mostly as many as corolla lobes, inserted near the base or at the medial zone or near the orifice of corolla tube; anthers included or exerted, oblong to narrowly elliptic to button-shaped, 2-locular, opening by longitudinal slits, dorsifixed near the base or around the middle, introrse; pollen released as monads, colpate or colporate, exine reticulate or foveolate (not echinate); style branches present, with stigmatic surface smooth to verrucate; ovary inferior (half-inferior in *Rachicallis*), bilocular, with a few to many ovules (1 to 2 in *Mazaea*) per locule attached to a central placenta, or exceptionally one ovule per locule basally attached (*Phyllomelia*). Fruits woody capsules, loculicidal or septicidal, or septicidal and loculicidal contemporaneously (*Blepharidium*, *Mazaea*), commonly dehiscent basipetally, or exceptionally pseudosamaras, indehiscent (*Phyllomelia*); placenta central, rarely apically incomplete, or shortly stalked; seeds horizontal, imbricate, peltate, and vertical, minute, 3- to 5-angular or dorsoventrally convex, not winged, wing concentric or bipolar (*Blepharidium*, *Mazaea*), or basally inserted, ellipsoid-ovoid and fleshy (*Phyllomelia*).

Genera included: *Acrosynanthus*, *Acunaeanthus* Borhidi, Komlodi & Moncada, *Blepharidium*, *Glionnetia* Tirveng., *Habroneuron* Standl., *Mazaea*, *Phyllomelia*, *Rachicallis*, *Rogiera*, *Roigella*, *Rondeletia*, *Rovaeanthus*, *Spathichlamys* R. Parker, *Stevensia*, *Suberanthus*.

The description and delimitation of the Rondeletieae here proposed are based on the results of the present study in combination with those of Rova (1999) and Rova et al. (2002). The description is basically a reduction of that proposed by Delprete (1999a), based on his wide circumscription of the tribe to include the Condamineeae and the Sipaneeae, which was produced primarily for the floristic treatment and not based on a comprehensive phylogenetic analysis.

Rova (1999) and Rova et al. (2002) demonstrated that the Condamineeae (except the subtribe Portlandiinae, which belongs to the Chiococceae s.l.) should be transferred to the subfamily Ixoroideae, in a complex also including the Calycophylleae and the Hippotideae (more studies are needed to re-delimit these groups; Kainulainen & Bremer, unpublished). Delprete and Cortés-B. (2004) and Rova et al. (2002) also demonstrated that the Sipaneeae belongs to the subfamily Ixoroideae and is a monophyletic group that was positioned in the same clade as the tribes

Henriquezieae and Posoquerieae in their phylogenetic analysis (Delprete et al., 2004).

The monotypic genus *Rachicallis*, endemic to the Caribbean Basin, is added (not included in the tribe by Delprete, 1999a) to the present delimitation of the Rondeletieae, which was placed close to this tribe in Bremer et al. (1995) and shown to belong to Rondeletieae by Rova et al. (2002) and in the present study.

As a result of this study, *Stevensia* is perhaps best treated as synonymous with *Rondeletia*, because in the phylogenies obtained it is positioned within the *Rondeletia*. However, as only one species of *Stevensia* (*S. minutifolia*) was included in the analysis, we refrain from proposing the necessary new combinations.

TAXA TRANSFERRED TO THE TRIBE GUETTARDEAE

Steyermark (1964) positioned *Cuatrecasasiodendron* in the Rondeletieae because of its foliaceous calyx lobes, capsular fruits, horizontal seeds, ovary with many ovules in each locule, and corolla with imbricate lobes. At the same time, he treated it as closely related to *Rondeletia* because of the corolla lobes being subzygomorphic, as the most interior lobe is more pubescent internally than the external ones, and glabrous or almost glabrous externally, while the others are pubescent externally. This genus was maintained in the Rondeletieae by Delprete (1999a) because of the same characters as used by Steyermark. However, in the phylogenies produced in the present study, *Cuatrecasasiodendron* was found within the *Arachnothryx* clade of the tribe Guettardeae, and the two taxa are treated here as synonymous.

In addition, a detailed analysis of the two species of *Cuatrecasasiodendron* described by Steyermark was undertaken. Steyermark (1964) distinguished *C. spectabile* Steyerm. from the type species because of its leaf blades hirsute below (vs. adpressed-pilose to arachnoid-pubescent below), shorter petioles, shorter stipules, corollas 17–20 mm long (vs. ca. 28 mm long, with longer pubescence), and longer and more secundiflorous inflorescence branches among other characters. A comparison of the type specimens with recent collections revealed that the characters used by Steyermark to separate the two taxa fall into a morphologic (and geographic) gradient.

The types of both taxa of *Cuatrecasasiodendron* were collected in the Valle del Cauca Department (Colombia); however, *C. spectabile* is from a low elevation of the coastal region, while *C. colombianum* Standl. & Steyerm. is from higher elevations of the Central Cordillera, and recent collections showed intermediate characteristics. Therefore, the two spe-

cies are treated here as synonymous to one another, and only one new combination in *Arachnothryx* is necessary.

Arachnothryx Planch., Fl. Serres Jard. Eur. 5: 442. 1849. TYPE: *Arachnothryx leucophylla* (Kunth) Planch. (= *Rondeletia leucophylla* Kunth).

Cuatrecasasiodendron Standl. & Steyerm., Acta Biol. Venez. 4: 29. 1964. Syn. nov. TYPE: *Cuatrecasasiodendron colombianum* Standl. & Steyerm.

Arachnothryx spectabilis (Steyerm.) Rova, Delprete & B. Bremer, comb. nov. Basionym: *Cuatrecasasiodendron spectabile* Steyerm., Acta Biol. Venez. 4: 33. 1964. TYPE: Colombia. Valle del Cauca Department: Costa del Pacifico, Río Cajambre, Barco, 5–80 m, 21–30 Apr. 1944 (fl.), J. Cuatrecasas 17165 (holotype, US!; isotype, VEN!).

Cuatrecasasiodendron colombianum Standl. & Steyerm., Acta Biol. Venez. 4: 30. 1964. Syn. nov. TYPE: Colombia. Valle del Cauca Department: Cordillera Central, Vertiente Occidental, Hoya del Río Achicayá, Quebrada El Retiro, 300 m, 19 Dec. 1942 (fl.), J. Cuatrecasas 13694 (holotype, F!; isotype, US!).

Additional specimen examined. COLOMBIA. **Depto. Valle del Cauca:** Mun. Buenaventura, rd. Queremal–Anchicayá, Km 35, ca. 03°37'N, 76°53'W, ca. 300 m, 9 Apr. 1994 (fl.), J. H. E. Rova, L. Andersson, C. Gustafsson & C. Persson 2093 (GB).

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